Density, fat, water and solids in freshly isolated tissues

T. H. ALLEN, H. J. KRZYWICKI AND J. E. ROBERTS
U.S. Army Medical Research and Nutrition Laboratory, Denver, Colorado

Fat, water and tissue solids of the whole body less its bone mineral

T. H. ALLEN, B. E. WELCH, T. T. TRUJILLO
AND J. E. ROBERTS
U.S. Army Medical Research and Nutrition Laboratory, Denver, Colorado

Reprinted from
JOURNAL OF APPLIED PHYSIOLOGY, Vol. 14, No. 6, November 1959
Printed in U.S.A.

Reprinted from JOURNAL OF APPLIED PHYSIOLOGY
Vol. 14, No. 6, November, 1959
Printed in U.S.A.

Density, fat, water and solids in freshly isolated tissues

T. H. ALLEN, H. J. KRZYWICKI AND J. E. ROBERTS[1]
U.S. Army Medical Research and Nutrition Laboratory, Denver, Colorado

ALLEN, T. H., H. J. KRZYWICKI AND J. E. ROBERTS. *Density, fat, water and solids in freshly isolated tissues.* J. Appl. Physiol. 14(6): 1005–1008. 1959.—Once the initial density, D_1, is noted, a single piece of freshly isolated mammalian tissue is analyzed for water and fat. Knowing the densities of water and fat, the tissue's fat-free density, D_2, and the dry, fat-free density, D_3, can be computed. Among the various types of soft tissues, since D_3 is similar, a simple relationship is shown to exist between the fat-free water content and the corresponding density. The biological extremes in a water:fat:protein system thus operate in such a way that the quantity of fat can be predicted from an equation containing three variables, i.e. the initial weight and density of a tissue and its quantity of water. This is of interest in human body composition and also to those who desire to make a sequence of determinations on a single piece of tissue without having to submit a portion thereof to direct analysis of fat, e.g. to express tissue electrolytes and water content on the fat-free basis.

MUCH CAN BE LEARNED from the relationships among density, fat and water in a wide variety of tissues. Fatty tissue has low density, much fat, little water and scarcely any fat-free solids. This tissue has a density like that of extracted fat (1), except for the influence of admixture with water and solids. Other soft tissues show an excellent mathematical relationship between the water content and the reciprocal densities when these are stated on a fat-free basis. Hence, it becomes possible to derive a single equation to predict the fat from the observed tissue densities and weights of water. This pertains to all soft tissues examined, including fatty tissue. The versatility of this equation will be verified, with the view in mind of applying it to human body composition.

METHODS

Density, water, fat and nitrogen. Subcutaneous, inguinal, omental and perirenal fatty tissues were supplied by the Surgery Division, Fitzsimons Army Hospital. These were removed, while subject was under general anesthesia, early in the course of surgery performed on nine patients requiring correction of a hernia, cholecystectomies, gastric resections, osteotomy, vagotomy, and adrenalectomy or oophorectomy in two cancer patients. The tissues were wrapped in plastic sheets and stored in a closed vessel to retard loss of water during the immediately following hour when tissue densities were measured. Other soft tissues were removed by dissection of bled-out rats, guinea pigs and rabbits. The tendons were ox Achilles bursa and rattail. The skeletal muscles were the gastrocnemius and the biceps femoris. Guinea pig and rabbit brain was separated into cerebral hemispheres and the brain stem.

The tissues were cut into pieces of from 0.4 to 25 gm in size, suspended on a thin steel hook, immediately weighed to the nearest milligram in air and then immersed to a fixed depth in kerosene or Skellysolv B of measured density. These liquids were either room temperature and close to 27°C or else were heated to 37°C by means of water pumped from a constant temperature bath through a coil of copper tubing. Since these liquids are immiscible with water, the tissue weight did not change perceptibly while immersed. Air bubbles do not collect on the tissues, e.g. among the clipped hairs in skin. Each tissue sample was transferred to a tared, steel dish and placed in a vacuum drying oven at 50°C for 3 days or longer to get the weight of water by difference. The thoroughly dried tissues on the tared dishes were extracted with daily changes of ethyl ether for 3 days before obtaining the weight of fat by difference.

MATHEMATICAL RELATIONSHIPS

This study requires the formal development of relations involving fundamental parameters and variables. These occur in equations which are stated explicitly in terms of the masses and densities of primary components of tissues. The actual determinations of tissue mass and density establish the numerical values of the parameters and further reveal that diverse tissues behave like a physical system of simple admixture.

Consider the weight of a freshly isolated tissue to be composed of fat, water and residual mass, i.e. $M_1 = F + W + M_3$. Designate the fat-free weight as $M_2 = M_1 - F$ so that the corresponding density becomes

$$D_2 = \frac{d_f D_1 M_2}{d_f M_1 - D_1 f} \qquad (1)$$

Received for publication June 15, 1959.

[1] Present address: Department of Medicine, University of Colorado School of Medicine, Denver, Colo.

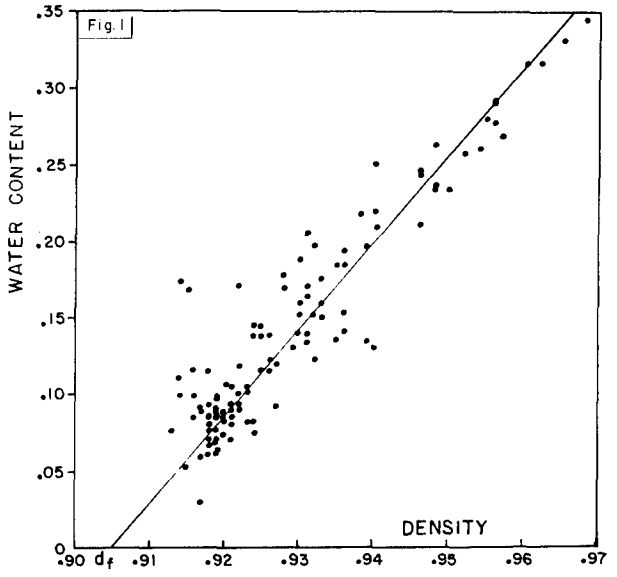

FIG. 1. Density and water content observed in human fatty tissue at 27°C. Curve is drawn according to *eq. 4* discussed in text.

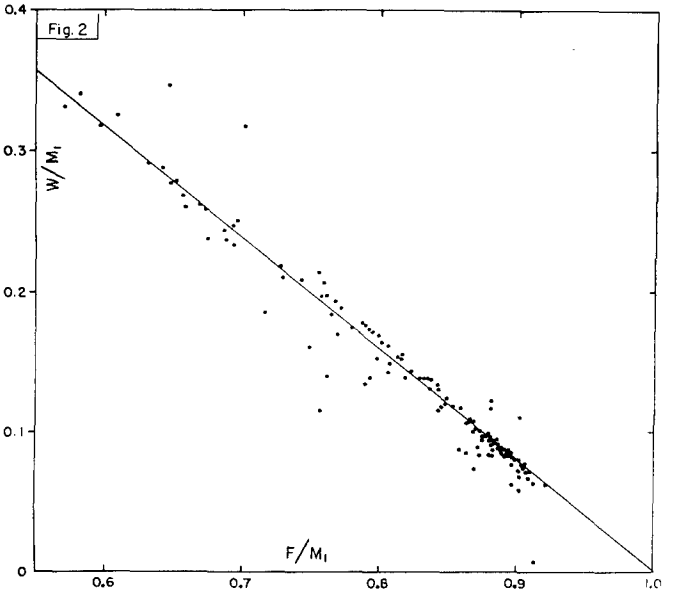

FIG. 2. Constancy of water content in human adipose tissue. Slope and intercept on the W/M_1 axis show $\delta = 0.792$ with $\sigma_\delta = 0.013$.

where D_1 is the observed density of the tissue and d_f is the density of neutral fat as found by Fidanza *et al.* (1). The fraction of water contained within M_2 is denoted,

$$\delta = W/M_2 \qquad (2)$$

It is extremely convenient to establish a simple relationship between D_2 and δ. Note that $M_2 = M_3 + W = V_3D_3 + V_wd_w$, which can be solved to give $V_w = V_2[(D_3 - D_2)/D_3 - d_w)]$. Since $\delta = V_wd_w/M_2$, by substituting for V_w, the resulting equation can be written in the form

$$\delta = \frac{d}{D_2} - a \qquad (3)$$

where $a = d_w/(D_3 - d_w)$ and $b = D_3d_w/(D_3 - d_w) = aD_3$.

The quantity of fat can be stated in terms of M_1, D_1, and W by solving *equation 3* for D_2 and equating with *equation 1* whereupon,

$$F = K\left[M_1\left(\frac{b}{D_1} - a\right) - W\right] \qquad (4)$$

where $K = [(b/d_f) - a]^{-1}$.

RESULTS

Adipose tissues. Some of these tissues are so extremely rich in fat that the tissue density approaches the density of extractable fat (fig. 1). This is particularly true in tissues from obese persons, just as with Pitts' guinea pigs (2). The mean $d_f = 0.905$, and the 1σ range along the abscissa is 0.0067, showing that there is no single absolute value for the density of human fat. Obviously, there is a wide range in water content and in tissue density. However, these variables appear to be related. In fact, the curve shown in figure 1 was drawn by means of *equation 4*. It will be shown below that the parameters in this equation can be designated according to the densities of fat, water and the residual mass. This implies that the fat-free portion of adipose tissues belongs to the same water: density system as the other fat-free soft tissues of the body.

Whereas notable scatter occurs in the data about the

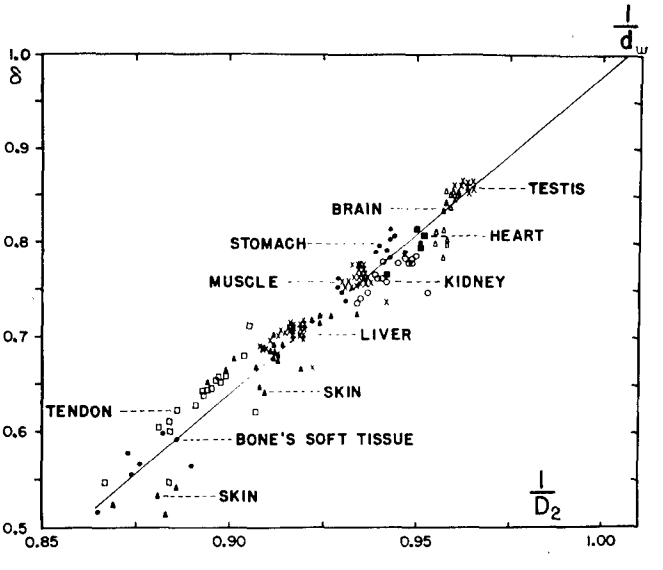

FIG. 3. Verification of the constants a, the intercept, and b, the slope, in *eq. 3* as based on 155 tissue determinations at 27°C.

TABLE 1. *Prediction of Fat-Free Weight of Tissue Samples**

Tissue	Temp., °C	N	Dev., %	$\sigma_{Dev.}$, %	δ	D_2	D_3
Testis	27	10	−0.50	0.54	0.858	1.038	1.39
	27	8†	−0.25	0.83	0.858	1.036	1.36
	37	39	−0.75	1.24	0.862	1.039	1.45
Brain	27	9	−2.74	0.55	0.838	1.043	1.37
	27	9†	−0.04	1.17	0.846	1.041	1.37
	37	18	+0.60	1.36	0.836	1.043	1.40
Heart	27	4	+0.63	0.78	0.795	1.054	1.36
	37	21	+0.67	1.40	0.789	1.058	1.40
Adipose, human	27	122	−0.84	2.42	0.795	1.057	1.38
Adipose, rodent	27	19	−0.74	2.52	0.718	1.083	1.39
Adipose, rodent	37	29	+0.53	2.29	0.746	1.073	1.40
Stomach	27	12	−0.54	1.02	0.777	1.065	1.40
and duodenum	27	10†	−0.93	2.83	0.781	1.064	1.40
Spleen	27	4	−0.75	0.26	0.773	1.067	1.40
	27	4†	+1.02	1.32	0.773	1.069	1.42
	37	21	−1.51	1.52	0.781	1.063	1.42
Kidney	27	10	+0.97	0.57	0.760	1.064	1.35
	27	12†	−1.78	1.88	0.754	1.064	1.35
	37	43	+0.65	1.19	0.775	1.061	1.39
Muscle	27	20	−0.37	0.84	0.765	1.068	1.39
	27	21†	+0.09	0.87	0.760	1.070	1.39
	37	39	+0.17	1.23	0.765	1.066	1.49
Liver	27	27	−0.64	0.59	0.700	1.092	1.40
	27	19†	−0.74	1.28	0.703	1.084	1.37
	37	17	+1.52	2.12	0.707	1.081	1.37
Skin	27	25	−0.33	1.53	0.654	1.103	1.38
	37	105	+1.39	2.34	0.679	1.088	1.37
Tendon	27	19	−1.90	1.40	0.619	1.123	1.41
	37	32	+0.67	1.75	0.621	1.115	1.39
Bone less mineral	26	8	+0.09	1.45	0.567	1.135	1.39
Total		736					
Mean			−0.062	1.777			1.389

* Using *eq. 4*, the corresponding water content and densities and the mean densities of dry, fat-free tissues. † Deprived of food and water.

curve in figure 1, the same tissue samples have marked uniformity of water content on the fat-free basis, as demonstrated by means of figure 2. Note from *equation 2* that $W = \delta M_2 = \delta(M_1 - F)$, and that division by M_1 yields $W/M_1 = \delta - \delta F/M_1$. The data are plotted in exactly this form in figure 2, such that δ is defined by both the slope and the intercept ($F = 0$) of this line. Thus, $\delta = 0.792$. Since $\sigma_\delta = 0.013$, δ operates virtually as a constant for this tissue. Hence, if human fatty tissue weight and its water weight are known, by using $F = M_1 - W/\delta$, the fat can be estimated to within 1% of the original weight.

Other soft tissues. Nonfatty soft tissues make up the bulk of the body and lend themselves to a solution of the chief problem now posed by *equation 3*, and consequently *equation 4*. Providing *equation 3* is valid, the plot of δ and $1/D_2$ should yield a straight line with slope b and intercept a. By accepting $d_f = 0.911$ for fat from all tissues (1) except brain lipid, which here was found to have $d_f = 1.026$ at 27°C, values of D_2 (*eq. 1*) were obtained from tissues of well-fed animals. The straight line through these data (fig. 3) was established by the method of averages (3), giving a slope, $b = 3.401$, and an intercept, $a = -2.423$. As a check of these values, when $\delta = 1$, $D_2 = 0.9936 = d_w$, the density of water at 36°C. Testis, muscle and liver have notably fixed values of $1/D_2$ and δ, whereas some of the viscera and especially belly, flank and back skins, and tendons have considerable ranges in δ in direct proportion to ranges in $1/D_2$. This indicates that falling water content is accompanied by increased densities in nonfatty tissues.

The accuracy of *equation 4* was tested by comparing the predicted fat-free weights with those actually found by extraction. The differences (table 1) are expressed as the mean deviations in percentage of the fresh weights of all tissue samples examined. The precision is shown by standard deviations of these differences. Some of the determinations were made on tissues from adult rats kept from food and water until the body weight had fallen by as much as one-third. Although the organs were much depleted, their tissues were well described by *equation 4*. The generally good agreement shows that the constants set forth in *equation 4* operate at different temperatures and in diverse nutritional conditions. Moreover, the soft tissue of guinea pig and rat femurs apparently belongs to the same protein:water:fat system as the other soft tissues (to be published).

The so-called dry solids, M_3, contributing to the original density of a tissue, are chiefly proteins together with lesser quantities of small molecular solutes. The denoted mass, $M_3 = M_2 - W$, has a density, $D_3 = (1 - \delta)/[(1/D_2) - (\delta/d_w)]$, for which the mean density originally imparted to each tissue sample therefore is 1.39 (table 1, final column). This is similar at 27°C and 37°C and is like that of adequately isolated proteins. Thus, G. S. Adair's results (4) show the density of ox carboxyhemoglobin to be close to 1.30 when dissolved at 1°C in dilute sodium chloride solution containing phosphates. Bear (5) reports that collagen attains densities as high as 1.41.

DISCUSSION

Except for a study of adipose tissue in two men undergoing weight loss (6), the above information appears to be the first complete account of the density of various, freshly isolated tissues for which the fat, water and solids of each sample were also determined and shown to contribute to the original density. This, together with a similar study of fresh bone and the density of its bone mineral (to be published), demonstrates the adequacy of Siri's concepts (7) as to the density of the whole body in terms of the proportions of its major constituents. In line with his notations, one can consider the total proportion of soft tissues of the whole body, $1 - m = w + f + s$, as having a density

$$\frac{1}{d_t} = \frac{w}{d_w} + \frac{f}{d_f} + \frac{s}{d_s}$$

Now, attempting to criticize, among the various tissues only the brain had a value of d_f decidedly larger than that currently assumed to hold for the whole body (1). Ideally, f/d_f should be expanded to account for brain lipid. According to an excellent compendium (8), the adult brain weighs about 1.4 kg and could contain 140 gm of lipid. Taking $d_f = 0.901$ instead of 1.002 for the density of brain lipid at body temperature, the weight of brain lipid is 127 gm. The error of 13 gm thus introduced is very small considering the precision of the present body water and density methods. Thus, f/d_f currently is acceptable when $d_f = 0.901$ gm/ml. The density of human fatty tissues varies to such an extent that $\sigma_{d_f} = 0.0067$, which is slightly larger than Fidanza *et al.* (1) found with extracted, neutral fat from internal sites.

An evaluation of s/d_s, according to the present results, shows that $D_3 = d_s \cong 1.39$ gm/ml. Included here are the small molecules and ions of solute along with the proteins of dried, defatted tissues. Do the solutes contribute much to the density of the so-called tissue solids? Perhaps; the small molecular solute is 1/100 of the tissue water. By assigning a density of 2 to these materials, it then follows that the density of the mixed proteins of the tissues is close to 1.37. Hence, the small solutes contribute but slightly to $d_s = 1.39$. The D_3 value for each of the nonfatty tissues (table 1) was separately computed, and by combining these it was found that $\sigma_{d_s} \cong 0.05$. Although it is interesting to suppose that d_s of a given tissue differs from that of another tissue, it is difficult to prove this convincingly. By pooling the results on the two tissues which differ the most (table 1), it appears, according to statistical methods (9), that the d_s of testis is distinctly greater than that of the kidney. This may reflect *in situ* differences in the chemical constitution of testicular and renal proteins. However, these organs are only a small though an admittedly valuable part of the body, most of which is muscle, skin and other viscera (8). Therefore, for purposes of whole-body composition, $d_s = 1.399$ and $\sigma_{d_s} = 0.051$ gm/ml at body temperatures. To refine and expand this into diverse components requires more numerous, detailed analyses of each fresh tissue sample than presently attempted.

The tissue water is believed to have been measured accurately. This was done by drying, at a temperature of 50°C in a vacuum oven through which a stream of dry air was admitted, until after 3–5 days the tissues had reached an ascertained constant weight. Each type of tissue had characteristic proportions of water. Since d_s operates as a constant, it therefore follows that fat-free tissue water content should vary inversely with the fat-free tissue densities (table 1). This was readily verified for all except the fatty tissues. In that instance, the precision of stating the density of the neutral fat was insufficient to permit the derivation of the fat-free density from the initial density of single pieces of fatty tissue. However, the relationship between the weights of water and the weights of fat-free human adipose tissue had considerable uniformity. With adipose tissues, densitometry is insufficient for accurate prediction of the fat. In order to achieve reasonable precision, the densitometry therefore needs to be combined with measurements of water content. When this is done, the fat-free weight of the various tissues can be predicted from *equation 4* with good accuracy and with a precision for a single determination of 1.78% of the fresh weight (table 1, final line). Contained within *equation 4* are the constants for the densities of water, fat and the tissue solids. These constants have been ascertained as operating smoothly between the biological extremes of one or more components in the water:fat:solid system in freshly isolated tissues, ranging from brain to soft tissue in bone.

C. F. Stewart and J. A. Evans, Jr. assisted greatly in many of these determinations.

REFERENCES

1. Fidanza, F., A. Keys and J. T. Anderson. *J. Appl. Physiol.* 6: 252, 1953.
2. Pitts, G. C. *Am. J. Physiol.* 185: 41, 1956.
3. Lipka, J. *Graphical and Mechanical Computation.* New York: Wiley, 1918, p. 124.
4. Adair, G. S. *Haemoglobin*, edited by F. J. W. Roughton and J. C. Kendrew. New York: Interscience, 1949, p. 185.
5. Bear, R. S. *J. Biophys. & Biochem. Cytol.* 2: 363, 1956.
6. Entenman, C., W. H. Goldwater, N. S. Ayres and A. R. Behnke, Jr. *J. Appl. Physiol.* 13: 129, 1958.
7. Siri, W. E. *Adv. biol. med. Phys.* 4: 239, 1956.
8. Roessle, R. and F. Roulet. *Mass und Zahl in Pathologie.* Berlin: Springer, 1932.
9. Youden, W. J. *Statistical Methods for Chemists.* New York: Wiley, 1951.

Reprinted from JOURNAL OF APPLIED PHYSIOLOGY
Vol. 14, No. 6, November, 1959
Printed in U.S.A.

Fat, water and tissue solids of the whole body less its bone mineral

T. H. ALLEN, B. E. WELCH,[1] T. T. TRUJILLO[2]
AND J. E. ROBERTS[3]
U.S. Army Medical Research and Nutrition Laboratory, Denver, Colorado

ALLEN, T. H., B. E. WELCH, T. T. TRUJILLO AND J. E. ROBERTS. *Fat, water and tissue solids of the whole body less its bone mineral.* J. Appl. Physiol. 14(6): 1009-1012. 1959.—Except for bone mineral, the body is shown to belong to the same water:fat:protein system as its soft tissues. Hence, an equation verified with a variety of freshly isolated tissues can be used to estimate the body fat and the so-called total tissue solids. On the average, there are 0.784 kg of water/kg of body weight less bone mineral and fat. However, this water content probably fluctuates between extremes of 0.816 and 0.752, in accordance with the time elapsing since imbibing much water. This causes the density of the tissues in the fat-free, bone mineral-free body to range from 1.050 to 1.071. Combined simultaneous measurements of water, density and bone mineral, therefore, are required for the estimation of fat and tissue solids. Bone mineral occurs in the proportion of about one part to three parts of tissue solids, irrespective of ranges in quantities of fat and water among 30 healthy persons.

THE FIRST SUITABLE MEASUREMENTS of human body density (1) gave substantiation to postulations on the 'lean body mass' (2). Some important details pertaining thereto were described by Morales *et al.* in 1945 (3) and again in 1958 (4), in reply to questions which had been evoked by the growing interest, criticism and appreciation of Behnke's approach to body composition.

We have sought to verify the operation of the constants in the body composition equations from measurements performed on numerous tissues, including human fatty tissue (5) and bone (6). The soft tissues conform fairly well with a precise mathematical relationship between the water content and the densities, when stated on a fat-free basis. As shown below, this information can be applied to the intact human body. To do this, one first estimates the bone mineral (6), and then its effects on whole-body density and water content are removed from consideration. Thus, the bulk of the body is viewed as a mixture of soft tissues consisting of a water:fat:protein system which is shown to operate between extremes almost according to the manner deduced by Siri (7).

Received for publication June 15, 1959.

[1] Present address: Department of Physiology-Biophysics, USAF School of Aviation Medicine, Brooks Air Force Base, Tex.

[2] Present address: Biomedical Research Division, Los Alamos Scientific Laboratory, Los Alamos, N. Mex.

[3] Present address: Department of Medicine, University of Colorado School of Medicine, Denver, Colo.

METHODS

The following measurements of each subject were begun within a period of 2 hours or less.

Weight and density. Body weight to the nearest 10 gm, as noted with a calibrated Plima scale, was taken when the bladder and bowels had been evacuated following an overnight fast. Weighing under water was performed three times on each subject, as described by Welch and Crisp (8), who have introduced a slight correction for the depth under water. The mean observed density was corrected further by adding 0.001 gm/ml for air judged to present in the gastrointestinal tract (9). The men were nude. The women wore undergarments, bouyant to 10 gm.

Bone mineral estimation. Height and joint diameters were noted to the nearest millimeter, the subject being erect and barefoot. The intercondylar dimensions at the elbows, wrists, knees and ankles (10) were measured with machinist's calipers, the jaws of which were fastened tightly in place and then removed and laid along a millimeter rule to sight for distance. The estimated weight of bone mineral is $m = 3.9(10^{-1})(H)(\bar{T}^2)$ where height, H, and the mean transverse diameter, $\bar{T}$, are in centimeters (6). The density of bone mineral is $d = 2.8$ (6).

Body water. Deuterium oxide (50 ml) and tritium-enriched water (5 ml, 3.7 mc) were ingested simultaneously and followed with 450 ml of hot tea, after which nothing was drunk for 5 hours (11). Four subjects received HTO only. The Consolidated Electrodynamics Corporation model 21-620 mass spectrometer was used to measure HDO concentrations in serum and urine water (11). HTO in urine was measured by a liquid scintillation method (12). The weight of body water was computed as that immediately prior to ingestion of the water tracers (11).

TABLE 1. *Estimates of Bone Mineral and Kilogram Quantities of Water, Fat and Dry Solids**

m	M_1	D_1	W_{HDO}	W_{HTO}	F†	F‡	M_2†	M_2‡	M_3†	M_3‡
					Men					
3.64	52.82	1.018	34.4	(36.8)	10.4	(7.2)	42.4	(45.6)	8.0	(8.8)
3.16	60.76	1.006	34.4	35.9	17.6	16.5	43.2	44.3	8.8	8.4
3.89	61.94	1.014	39.5	41.3	13.4	12.1	48.5	49.8	9.0	8.5
3.35	59.50	1.014	37.5	36.6	13.2	13.9	46.3	45.6	8.8	9.0
4.12	64.79	1.015		41.8		13.5		51.3		9.5
3.75	61.00	1.016	36.8	37.8	14.5	13.7	46.5	47.3	9.7	9.5
3.31	60.57	1.018	35.2	37.5	15.1	13.4	45.6	47.2	10.4	9.7
3.12	52.34	1.033		32.2		9.8		42.5		10.3
3.78	65.73	1.018	39.2	40.8	15.7	14.5	50.0	51.2	10.8	10.4
3.47	71.30	1.007	37.6	38.8	22.5	21.6	48.8	49.7	11.2	10.9
3.64	71.49	1.010	40.3	40.8	20.1	19.7	51.4	51.8	11.1	11.0
3.22	61.25	1.025	36.0	36.9	13.9	13.2	47.4	48.1	11.4	11.2
3.10	62.57	1.022	35.8	37.0	15.4	14.5	47.2	48.0	11.4	11.1
4.29	79.91	1.000	39.9	42.3	28.3	26.5	51.6	53.4	11.7	11.1
3.89	70.48	1.017	42.6	41.9	16.5	17.0	54.0	53.5	11.4	11.6
3.82	61.65	1.034	41.4	40.5	8.9	9.5	52.8	52.2	11.4	11.7
4.24	65.11	1.037	44.2	44.3	8.6	8.5	56.5	56.6	12.3	12.3
4.14	73.71	1.017	41.7	41.5	19.4	19.5	54.3	54.2	12.6	12.7
4.43	87.56	1.006	46.0	49.1	28.0	25.7	59.6	61.9	13.6	12.8
4.16	73.65	1.025	43.1	45.1	16.8	15.4	56.9	58.3	13.8	13.2
4.42	93.53	1.005	46.9	52.5	31.8	27.6	61.7	65.9	14.8	13.4
4.51	74.40	1.038	49.6	50.4	10.3	9.7	64.1	64.7	14.5	14.3
3.63	118.01	0.991	(48.2)	55.8	(52.3)	46.7	(65.7)	71.3	(17.5)	15.5
					Women					
2.72	53.67	1.005		28.6		17.0		36.7		8.1
2.85	56.27	1.010	33.7	32.7	14.3	15.1	42.0	41.2	8.3	8.5
2.63	54.80	1.008	30.3	29.2	16.1	16.9	38.7	37.9	8.4	8.7
2.83	58.81	1.009	32.8	32.1	17.0	17.5	41.8	41.3	9.0	9.2
2.99	61.49	1.006	33.0	31.7	19.1	20.1	42.4	41.4	9.4	9.7
3.25	68.05	1.002	35.4	36.3	22.7	22.0	45.4	46.1	10.0	9.8
3.15	66.96	1.016		37.4		18.1		48.9		11.5

* In 30 men and women, on the basis of relation between M_1, D_1 and W by HDO and HTO methods. Parentheses enclose results believed to be dubious because of possible error in W. † Using D_1 and W by HDO. ‡ Using D_1 and W by HTO.

Equations. The body weight consists of bone mineral, fat, water and a residual mass. $M = m + F + W + M_3$. Other masses are defined as: $M_1 = M - m$; $M_2 = M_1 - F$; $M_3 = M_2 - W$. Having noted the density of the whole body, the density of the body less bone mineral is clearly $D_1 = (M - m)/(V - v)$ which can be rewritten:

$$D_1 = \frac{dDM_1}{dM - Dm} \qquad (1)$$

Studies of isolated tissues showed that the major constituents of the body resemble a physical system of simple admixture. The following equation was derived and verified:

$$F = M_1\left(\frac{2.516}{D_1} - 1.793\right) - 0.740\ W \qquad (2)$$

where the numerical values of the constants are based on the densities of fat, water and a dried, defatted residual mass (5).

RESULTS

The observed densities and content of body water in 30 human beings are plotted in figure 1 along the dashed curve. When extended to the abscissa, this curve fails to describe the fatty tissues (5), and far too low a value is obtained for d_f, the density of fat. What causes the whole body not to match its fatty tissues? Obviously, the body contains a material of high density and low water content, whereas this material is absent from the soft tissues. Bone mineral can be corrected for as proposed above. Once this is removed from consideration, the body water is contained in a mass, smaller by 2.5–4.5 kg and of lower density. The data points corresponding to these corrected water content and densities are scattered along the curve which can be seen to describe both fatty tissue (5) and the body less bone mineral. This curve was drawn by utilizing *equations 3* and *4* of the preceding study (5) and also the mean δ value of the present subjects. The acceptance of this δ value accomplishes a weighting of the δ and D_2 values of the body's individual soft tissues, which values lie along the curve ranging between d_w and d_s. The intersection of the two curves gives the weighted values of the water content and density of the healthy body when free of fat and bone mineral. Note, when the water content is zero, the density, d_f, is that of neutral fat as shown by the intercept on the density axis.

Some quantitative aspects are given (table 1) under headings which show that estimates of fat differ to the extent by which there is variation between the measurements of body water by either method (11, 12). This also holds for M_2, which is the size of the body less its bone mineral and fat. When, from the later, the measured weights of water are deducted, the M_3 sizes usually agree to within a few tenths of a kilogram. Although the esthetic concept of a youthful lean body is lacking, it is interesting that M_3 is like a dried-out, defatted mass of protein together with traces of body solutes, glycogen, etc. This mass in the 30 human beings tested necessarily has a mean density of 1.40, as shown in the preceding report (5).

In 24 of the subjects the δ value, being the ratio of W to M_2 (5), is similar with either the HDO or the HTO methods. Among the entire 30 subjects the degree of hydration is also similar, as judged from a mean $\delta = 0.784$, with $\sigma_\delta = 0.016$. These healthy men and women therefore have a mean D_2 value of 1.061 (*eq. 3* (5)) which is the density of the body less bone mineral and fat. However, body density varies inversely with body water content (fig. 2) and appears to fluctuate in accordance with water intake and output. To show this, let us double the σ_δ and let it operate about a body-water size of 40 kg, whereupon ± 1.6 kg would be the fluctuation. Some would refuse this as being much too large a draught, yet, during a single day twice this quantity is usually drunk and taken with food. While the body maintains its average daily water balance, δ could fluctuate between extremes of 0.816 and 0.752 and cause D_2 to range from 1.050 to 1.071.

DISCUSSION

Actual determinations of density, water and fat in tissues were shown to permit the accurate estimation of

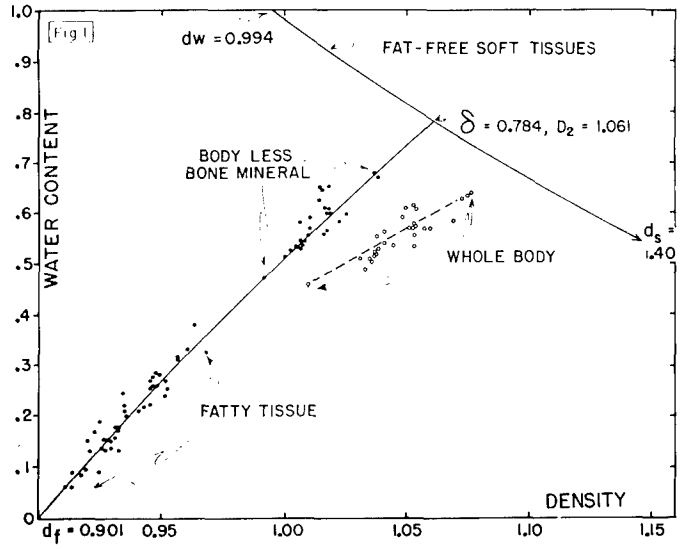

FIG. 1. Showing, once effect of bone mineral is removed, that a water:density system can be defined in terms of mean water content of the body's fat-free soft tissues. This water content of 0.784 gm/gm occurs at a fat-free, bone mineral-free density of 1.061 gm/ml, which is the intersection with the curve of fat-free soft tissues ranging between the density of water and the density of the so-called tissue solids.

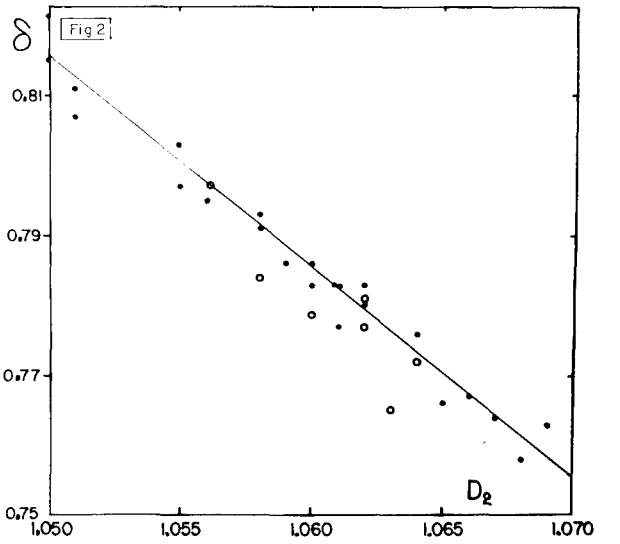

FIG. 2. Curve showing relationship between water content and body density on the bone mineral-free, fat-free basis. 23 men, *filled circles*; 7 women, *circles*.

fat from measurements of tissue density and water (5). The present results on human beings extend the tissue studies and show that correction for the high density of dry bone mineral (6) allows the body to match its soft tissues as to water, fat and solids. To predict a person's quantity of fat, therefore, requires measurements of bone mineral, body water and body density. The measurements of water and density should be done at the same time to avoid fluctuations in density caused by variation in body water, as from drinking and eating. Ideally, when the densitometry has been completed the water tracer is then taken. Although more than 3 hours often elapse before the HDO or HTO is thoroughly mixed, it is easy to state the weight of body water just prior to taking the water tracer (11). Behnke and Siri (13) are believed to be the only others reporting all of the necessary dimensions, including joint diameters from x-ray films. Although they measured the body density and water at different times when the body weight was not the same, their 22 sailors can be shown to have a mean $\delta = 0.784$ in exact agreement with our subjects. However, the variation is larger, as expected; σ_δ, being 0.029, is almost twice that presently observed.

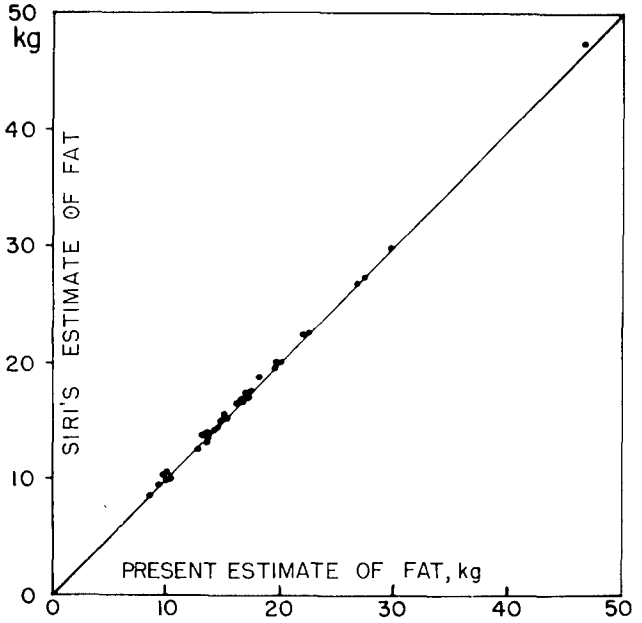

FIG. 3. Siri's equation is based on an assumed constancy between bone mineral and protein, whereas in our equation an actual estimate of bone mineral is introduced. However, good agreement occurs in the prediction of fat.

It is of interest to compare the estimates of fat by the present method with that using Siri's equation (7). As shown in figure 3 the agreement is remarkably good in healthy persons of either sex with quantities of fat in kilograms ranging from 8 to 48. On the average, his method estimates 40 gm more fat than ours; the variation between the two methods has a $\sigma = 287$ gm. Thus, Siri's original simplifying deductions can be shown to hold throughout a wide range in variation of the bone mineral:fat:water:solids of healthy persons. This occurs despite his using 3 instead of 2.8 for the density of bone mineral (6), 1.34 instead of 1.40 for the density of tissue solids (5) and a ratio of 5/12 for bone mineral to tissue solids.

It should be pointed out that M_3 is so defined as to include the soft tissue solids contained within the skeleton (6), which amounts to several kilograms of dry, fat-free solids. This lowers m/M_3 from 5/12, as implied by Siri, to 1/3, as can be noted from table 1. Is it also possible that bone mineral is a fairly uniform proportion of the fat-free body? Keys and Brozek (14), in citing analyses

of a few admittedly poor specimens of cadavers, were led to believe that about 7% of the fat-free body is ash. This agrees with the present results. The fat-free body is $L = M_2 + m = M_3 + \delta M_2 + m = M_3/(1 - \delta) + m = m/\gamma(1 - \delta) + m$. Hence, $m/L = \gamma(1 - \delta)/[1 + \gamma(1 - \delta)]$. Where $\gamma = m/M_3 = 0.337$ and $\delta = W/M_2 = 0.784$, the ratio of $m/L = 0.0679$. Therefore, it is reasonable to accept 6.8% of the normally hydrated, fat-free body as being composed of bone mineral, realizing that the 2-σ range lies between 5.7 and 7.9%.

These studies were made during October 1956 in the Los Alamos Scientific Laboratory by arrangements approved by Dr. T. L. Shipman and Lt. Col. L. M. Hursh. Dr. W. H. Langham made available the excellent facilities of the Biomedical Research Division. Their support of these studies is gratefully acknowledged. C. E. Crisp and P. E. Griffin performed numerous analyses of nitrogen for the calculation of lung volume at the moment of weighing under water. The HTO measurements were completed by T. T. Trujillo during our stay in Los Alamos. The HDO measurements were done later in Denver by A. D. Wentzel and P. L. Johnson.

REFERENCES

1. Behnke, A. R., B. G. Feen and W. C. Welham. *J.A.M.A.* 118: 495, 1942.
2. Behnke, A. R. *Harvey Lect.* 37: 198, 1941–1942.
3. Morales, M. F., E. N. Rathbun, R. E. Smith and N. Pace. *J. Biol. Chem.* 158: 677, 1945.
4. Morales, M. F. and A. R. Williams. *J. Appl. Physiol.* 12: 225, 1958.
5. Allen, T. H., H. J. Krzywicki and J. E. Roberts. *J. Appl. Physiol.* 14: 1005, 1959.
6. Allen, T. H. and H. J. Krzywicki. *Human Biology.* In press.
7. Siri, W. E. *Advanc. biol. med. Phys.* 4: 239, 1956.
8. Welch, B. E. and C. E. Crisp. *J. Appl. Physiol.* 12: 399, 1958.
9. Buskirk, E. R. *Human Biology.* In press.
10. Trotter, M. E. *Am. J. Phys. Anthropol.* 12: 537, 1954.
11. Wentzel, A. D., J. M. Iacono, T. H. Allen and J. E. Roberts. *Phys. in Med. Biol.* 3: 1, 1958.
12. Langham, W. H., W. J. Eversole, F. N. Hayes and T. T. Trujillo. *J. Lab. & Clin. Med.* 47: 819, 1956.
13. Behnke, A. R. and W. E. Siri. U.S. Navy Research and Development Technical Report TR-203 NS 080-001, Dec. 1, 1957.
14. Keys, A. and J. Brozek. *Physiol. Rev.* 33: 245, 1953.